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Time- and Dose-Dependent Changes in Neuronal Activity Produced by X Radiation in Brain Slices

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A new method of exposing tissues to X rays in a lead Faraday cage has made it possible to examine directly radiation damage to isolated neuronal tissue. Thin slices of hippocampus from brains of euthanized guinea pigs were exposed to 17.4 keV X radiation. Electrophysiological recordings were made before, during, and after exposure to doses between 5 and 65 Gy at a dose rate of 1.54 Gy/min. Following exposure to doses of 40 Gy and greater, the synaptic potential was enhanced, reaching a steady level soon after exposure. The ability of the synaptic potential to generate a spike was reduced and damage progressed after termination of the radiation exposure. Recovery was not observed following termination of exposure. These results demonstrate that an isolated neuronal network can show complex changes in electrophysiological properties following moderate doses of ionizing radiation. An investigation of radiation damage directly to neurons *in vitro* will contribute to the understanding of the underlying mechanisms of radiation-induced nervous system dysfunction.

INTRODUCTION

Although the nervous system is usually considered to be radioresistant, behavioral studies have shown decrements in performance and acute disorientation at doses of 5–10 Gy (*1, 2*). Ionizing radiation has been shown to modify neuronal activity both *in vivo* (*3–6*) and *in vitro* (*7–9*). Changes in blood pressure and blood flow, altered blood–brain barrier, and release of blood-borne mediators are likely to contribute to the observed damage *in vivo* (*10–16*). In an isolated preparation of neural tissue supplied with oxygen, glucose, and balanced salt solution, damage from ionizing radiation is likely to result from direct effects on the neurons and their microenvironment. By understanding the cellular changes produced by radiation, we can begin to address the mechanisms of the observed performance decrement.

A previous study on slices of hippocampus isolated from guinea pig brain (*7*) revealed that ^{60}Co radiation decreased the evoked synaptic response and decreased the ability of the synaptic potential to generate a spike. The decrease in the ability to generate a spike potential was not dependent on dose rate; a dose of 75 Gy was necessary to produce the effect at both 5 Gy/min and 20 Gy/min. On the other hand, synaptic damage was dose-rate sensitive. Fifty grays at 20 Gy/min produced damage equivalent to 100 Gy at 5 Gy/min.

The remote location of the cobalt source limited the time resolution and sensitivity of this earlier study. A more sensitive system was established by positioning an X-ray tube directly within a lead-lined Faraday cage (*17*). This allowed observation of electrophysiological parameters in a single slice of brain tissue, immediately before, during, and after exposure to ionizing radiation. This paper reports the effects of radiation from this X-ray system in hippocampal tissue.

METHODS

Slices (400–450 μm thick) of hippocampus were prepared from the brains of euthanized male guinea pigs as described previously (*7, 18–20*). A single slice was positioned in the recording chamber (0.5 ml volume) and constantly perfused (approximately 1 ml/min) with an artificial cerebrospinal fluid (aCSF) containing 124 m*M* NaCl, 3 m*M* KCl, 2.4 m*M* $CaCl_2$, 1.24 m*M* KH_2PO_4, 1.24 m*M* $MgSO_4$, 10 m*M* glucose, and 26 m*M* $NaHCO_3$, equilibrated with 95% O_2/5% CO_2 at 30 ± 1°C. Temperature was continually monitored and did not change with radiation exposure.

The characteristics of the X-ray system are described in detail elsewhere (*17*). Briefly, the X-ray source was a Kevex 50 kVp/1 mA unit with a molybdenum (Mo) target, beryllium window, and Mo filter (25 μm). This configuration provided a quasi-monoenergetic spectrum consisting primarily of 17.4-keV photons. Dose rate (1.54 Gy/min) was measured with a Capintec parallel-plate ionization chamber positioned at the location of the tissue. During experimental exposures, the chamber was removed and tissue was exposed for calculated time periods.

The hippocampal slice maintains a large degree of organization that can be observed easily through a dissecting microscope. Using visual cues and a knowledge of this organization, electrodes for stimulation and recording were positioned in defined locations (Fig. 1). A stainless steel concentric bipolar stimulating electrode was positioned in the stratum radiatum which contains afferents to the cells of interest (pyramidal cells) in the CA1 region of the hippocampus. About a millimeter away, a glass microelec-

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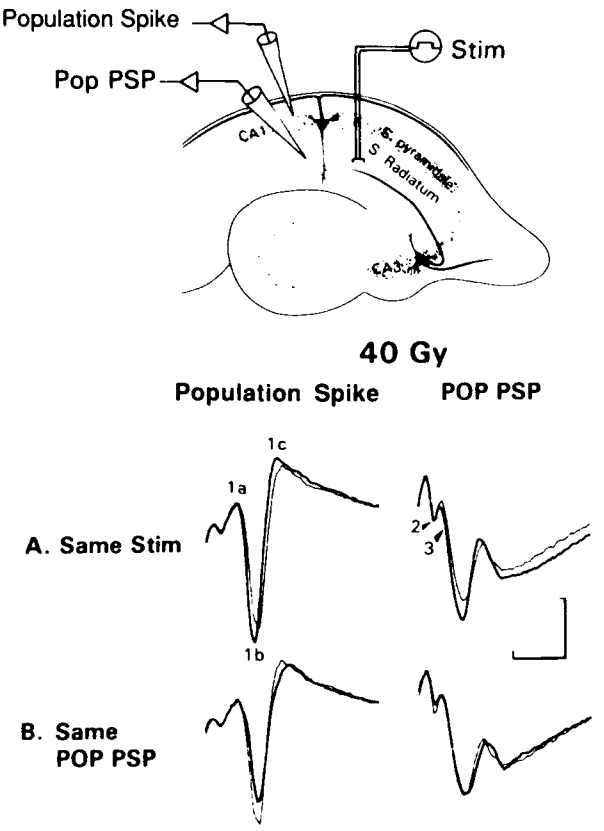

FIG. 1. Schematic diagram of the hippocampal slice preparation showing placement of electrodes. The stimulating electrode is in stratum radiatum of CA1. One recording electrode is in stratum radiatum of CA1 (pop PSP) and another is in stratum pyramidale of CA1 (population spike). Traces obtained from each of these recording sites are shown: On the left are recordings from the stratum pyramidale showing the summation of the responses of the CA1 neurons (population spike). Its amplitude is calculated from the values of the potentials at 1a, 1b, and 1c: 1/2(a + c) − b. On the right are recordings from the stratum radiatum showing the activation of the afferent fibers (afferent volley) at 2 and the summated response of a population of dendrites of the hippocampal neurons (pop PSP). The pop PSP is quantitated from the slope of the potential at 3. Recordings before and after exposure to 40 Gy X radiation (1.54 Gy/min) are superimposed. The thin line shows control traces and the thick line shows traces following exposure. (A) Using identical stimulus intensities to the afferent pathway produces the same size afferent volley but a larger pop PSP after radiation. The population spike is also larger. (B) If the stimulus intensity to the afferent pathway is reduced to produce a pop PSP of the same size as control, the afferent volley is smaller and the population spike following radiation is also smaller. Calibration pulse: 2 ms, 1 mV population spike, 0.5 mV pop PSP.

trode filled with 2 *M* NaCl also was placed in the stratum radiatum to record the afferent volley (the summation of the potentials from the stimulated afferent fibers) and to record the population postsynaptic potential (pop PSP; the summation of the synaptic responses of the population of neuronal processes in the region of the microelectrode). A typical recording from this microelectrode can be seen in Fig. 1 (traces on right). The afferent volley is the potential at the arrow labeled 2. The rest of the trace reflects the pop PSP. The pop PSP was quantified by measuring the maximal slope at the onset of the response (near arrow 3). A second NaCl-filled microelectrode was positioned in the stratum pyramidale of the CA1 region of the slice. Through this electrode, we recorded the population spike (the summation of synaptically evoked spike potentials of nearby neurons). Figure 1 also illustrates a typical population spike (traces on left). The amplitude was measured as the difference between the average of the potentials at points 1a and 1c less the potential at point 1b. The electrical potentials recorded from the hippocampal tissue were amplified by a high-gain extracellular amplifier and monitored on an oscilloscope. The data were digitized, stored, and analyzed with a PDP 11-73 computer.

Input–output (I/O) curves for the slice were obtained and analyzed as previously described (*7, 20*). Briefly, two series of 13 electrically isolated constant-current pulses (0.0 to 1.5 mA, 300 μs, 0.2 Hz) were provided to the stimulating electrode. The responses at the two microelectrodes were recorded. Two curves were plotted from the data. Afferent volley vs pop PSP provided an indication of synaptic efficacy, the ability of the afferent pathway to evoke a synaptic response. Population post-synaptic potential vs population spike amplitude provided an indication of the ability of the synaptic response to generate a population spike (spike generation).

Following placement of electrodes, the X-ray tube (Kevex) was positioned in its holder at a constant distance from the preparation (*17*) to provide a dose rate of 1.54 Gy/min of 17.4 keV X rays. The lead-lined Faraday cage was closed and the interlock system activated. Tissue was stimulated at 0.2 Hz with a stimulus intensity that produced a population spike of approximately half-maximal amplitude. Every 5 min five traces were averaged and the data stored. At 5 min before radiation exposure and 5 min and 30 min after termination of exposure, input–output curves were obtained. At doses of 40 Gy and less, another I/O curve was obtained approximately 60 min following the end of the exposure. Sham slices were examined intermittently throughout the series of experiments. In the sham slices, I/O curves were obtained and evaluated at time points similar to those used with irradiated tissue. Changes in irradiated tissue were referenced to these control curves. Sham slices changed very little with time, but some trends were evident: maximal population spike amplitude tended to increase while the pop PSP slope decreased slightly. Experiments were limited to a 2-h duration; beyond 2 h, some sham slices began to show decline, most commonly reflected as a severe decrease in the pop PSP size.

Statistical treatment of the I/O curves has been described previously (*7, 20*). For each experiment, the maximal amplitude during the control period was normalized for the population spike amplitude to 5 mV, pop PSP slope to 0.5 mV/ms, and afferent volley to 2 mV. Average maximal amplitudes of the raw data during control period were 4.7 ± 0.1 mV for the population spike, 0.73 ± 0.03 mV/ms for pop PSP, and 1.4 ± 0.1 mV for volley (n = 60 slices). For each stimulus intensity the data from 5 to 10 slices were averaged for each experimental condition (i.e., radiation dose) to provide a mean I/O curve. Two curves (experimental and control) were compared by evaluating the residual sum of squares for the sigmoid functions computer-fitted to the data points of the individual curves and the residual sum of squares for the function fitted to the data of both curves combined. Significance was accepted at $P < 0.05$. Differences between curves were quantified by comparing the ratios of the parameters of the computer-fitted functions.

RESULTS

Exposure of hippocampal slices to X radiation altered their electrophysiological properties. Figure 1 illustrates the changes in a slice exposed to 40 Gy. When the stimulus intensity was held constant, the afferent volley (arrow at 2) was unchanged by radiation exposure. Both the population spike and the pop PSP were increased in size. The increase in the population spike could result from the increased pop PSP and not be a direct effect of radiation. To test this, following irradiation the stimulus strength was decreased to

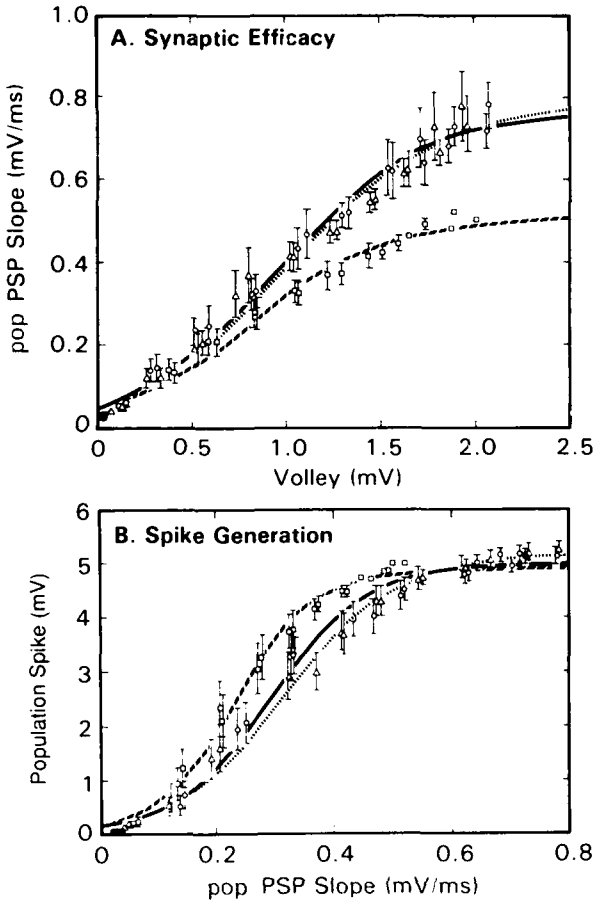

FIG. 2. Input-output curves averaged for six slices exposed to 50 Gy X radiation. Curves were obtained 5 min before exposure (squares; dashed line) and 5 (circles; solid line) and 30 min (triangles; dotted line) following termination of exposure. (A) Plot of afferent volley amplitude vs size of the pop PSP reflects the ability of the afferent pathway to generate a synaptic potential (synaptic efficacy). At all volley amplitudes, the pop PSP is larger following exposure to X radiation. (B) Plot of pop PSP vs population spike reflects the ability of the synaptic potential to generate a spike (spike generation). Following exposure to X radiation, a larger pop PSP is required to produce the same size population spike. Damage to spike generating mechanisms is more severe 25 min following exposure than 5 min following exposure.

produce a smaller afferent volley but the same size pop PSP (Fig. 1B). Under this condition, the population spike was smaller than prior to irradiation. This suggests that radiation has two effects: to increase the pop PSP and to decrease the ability of the pop PSP to generate a population spike.

The two effects can clearly be seen on the I/O curves for doses of 40 Gy and greater. The I/O curves for 50 Gy radiation are illustrated (Fig. 2). Plotting afferent volley amplitude vs the pop PSP size, the increase in the synaptic potential both 5 min and 30 min following exposure was evident (Fig. 2A). This reflects an increase in synaptic efficacy. Following exposure to 50 Gy the plot of pop PSP size vs the amplitude of the population spike was shifted to the right. This shift progressed with time (Fig. 2B). The pop PSP was less effective in producing a population spike. The I/O curves for 40 Gy (n = 6), 50 Gy (n = 6), and 65 Gy (n = 6) all showed similar changes.

One of the advantages of the X-radiation system used is that measurements of electrophysiological potentials can be made during the exposure. Throughout the exposure, afferents were stimulated at constant intensity. The pop PSP slowly began to increase. The increase progressed with time and continued following termination of exposure although at a slower rate. Following exposure, pop PSP size began to level off but recovery was not observed. Changes in the size of the pop PSP frequently became apparent within about 15 min, corresponding to a cumulative exposure of 23 Gy.

The time course of damage was also evaluated by obtaining I/O curves at two to three time points following termination of exposure to 5, 10, 20, 30, 40, 50, or 65 Gy. The curves for irradiated slices were compared to those for sham-irradiated slices obtained at similar time points in order to control for time-dependent changes not resulting from radiation exposure. Plotting the change in the I/O curves relative to the controls, one can see that the radiation damage progresses with time following exposure. In Fig. 3, data for 20, 30, 40, and 50 Gy are shown. No significant effects were observed with either 20 or 30 Gy. With 30 Gy, however, the trend is apparent. Forty and 50 Gy produced both a significant increase in synaptic efficacy and a significant decrease in the ability to generate the population spike. We observed no recovery during the time of the experiment.

Dose–response curves (Fig. 4) were constructed for the changes in synaptic efficacy and in spike generation from the I/O curves. The curves are plotted for the time point approximately 65–70 min following initiation of irradiation (circles). Also plotted (triangles) are the time points approximately 35–40 min following initiation of irradiation. Significant effects were seen at doses of 40 Gy and greater. The trend was apparent at 30 Gy. The effect at 35 min was always smaller than the effect at 65 min.

DISCUSSION

X radiation has been shown to increase synaptic efficacy and to decrease the ability to generate spikes at doses between 40 and 65 Gy. Synaptic efficacy progressively increases during exposure, with the first noticeable change occurring at a cumulative dose of approximately 25 Gy. Changes in both synaptic efficacy and spike-generating ability continue at a slower rate following exposure. The effects of radiation exposure persist following exposure until the termination of the experiment. Neuronal activity is altered through mechanisms that change the functional characteristics of individual cells without killing those cells.

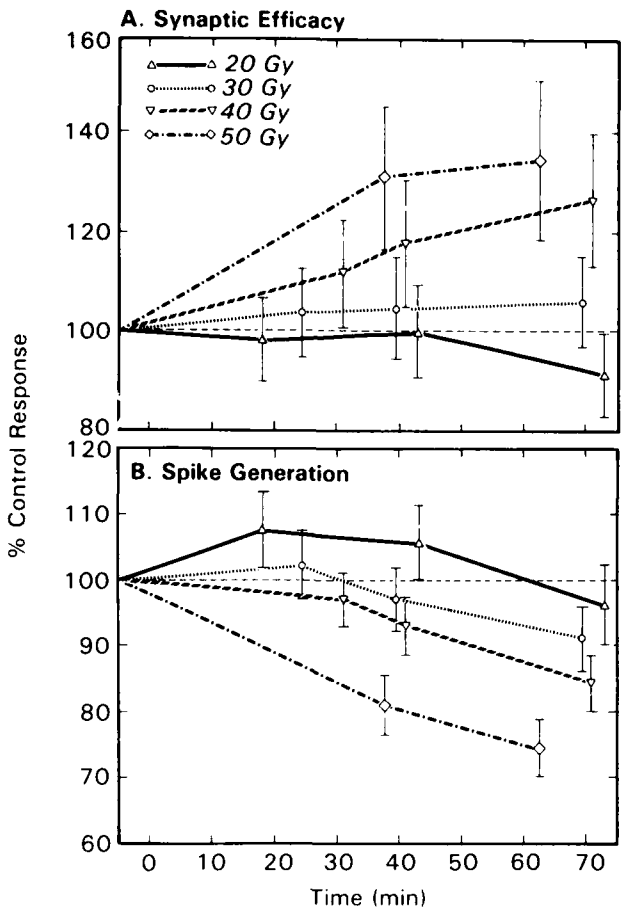

FIG. 3. Time course of radiation damage following exposure to 20, 30, 40, and 50 Gy X radiation. Input–output curves were obtained at the time points plotted. At each dose, the earliest time point was 5 min following termination of exposure. I/O curves were compared to sham-irradiated curves at similar time points. Irradiation was initiated at Time = 0 and terminated at Time = 13 min for 20 Gy, 19.5 min for 30 Gy, 26 min for 40 Gy, and 33 min for 50 Gy. (A) Changes in the curve relating afferent volley to pop PSP size reflect synaptic efficacy. There is no significant change with either 20 or 30 Gy but 40 and 50 Gy produce significant increases. (B) Changes in the curve relating pop PSP size to the population spike amplitude reflect changes in ability to generate spikes. Again, 20 and 30 Gy did not produce statistically significant changes but 40 and 50 Gy significantly depressed spike generation.

Neurons require generation of a spike to transmit their signal to the next cell in a pathway. If the synaptic potentials produced by stimulation of an afferent pathway are increased while the ability to generate a spike is decreased, the net output of the population of neurons may appear unaltered. Under normal circumstances, however, synaptic input to a cell is not a result of activation of an entire pathway. Rather, the summation of synaptic potentials from a number of discrete inputs is a complicated integrating process. This information processing could be severely compromised by seemingly minor changes. As a consequence, despite the 'balanced' changes in synaptic efficacy and spike generation, X radiation is likely to modify the functional properties of the hippocampus.

Both X radiation and γ radiation (7) produce deficits in spike generation. In the present study X radiation produced these changes at lower doses than γ radiation did (40 Gy rather than 75 Gy). One very important difference between the two studies is the difference in dose rate. Altering the dose rate from 5 to 20 Gy/min in the previous study did not alter the dose–response characteristics of the tissue for spike generation. A mechanism of lipid peroxidation was suggested as consistent with this observation. Lipid peroxidation is inversely dependent on dose rate at the lower dose rates but becomes relatively insensitive to dose rate at higher levels. This mechanism may also explain the greater sensitivity of the tissue in the present study using a dose rate of 1.54 Gy/min. Alternatively, the quality of radiation could be different enough to explain the effects. Experiments to distinguish these possibilities require a 17.4-keV

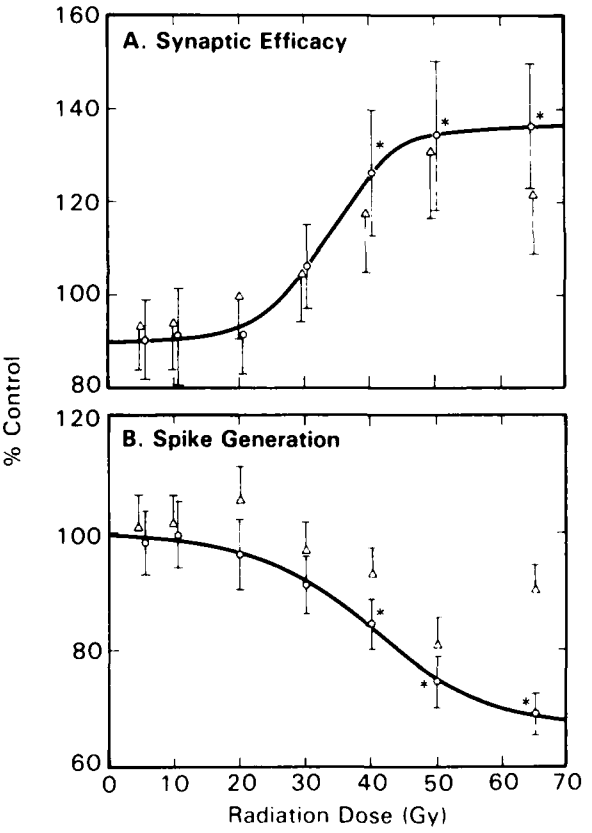

FIG. 4. Dose–response curves for electrophysiological damage to hippocampal tissue following exposure to X radiation. Data from time points 65 to 70 min following initiation of radiation exposure were plotted (circles). Triangles represent response at a time point 35–40 min following initiation of irradiation. (A) Dose–response curve constructed from the changes in the I/O curves relating afferent volley to pop PSP size, reflecting synaptic efficacy. (B) Dose–response curve constructed from the changes in the I/O curves relating pop PSP size to population spike amplitude, reflecting spike generation. Control, $n = 11$; 5 Gy, $n = 7$; 10 Gy, $n = 8$; 20 Gy, $n = 10$; 30 Gy, $n = 6$; 40 Gy, $n = 6$; 50 Gy, $n = 6$; 65 Gy, $n = 6$.

X-ray machine capable of providing higher dose rates to the slice preparation. Development of this system is in progress.

The present study demonstrates that X radiation can increase synaptic efficacy. This is in contrast to the γ-radiation studies where synaptic efficacy was reduced (*7*). Again, as with spike generation, the differences in dose rate and in radiation quality between these two experiments needs to be considered and evaluated in future experiments. A dose-rate effect seems to be a plausible explanation. At 5 Gy/min a greater dose of γ radiation is required to reduce synaptic efficacy than at a rate of 20 Gy/min (*7*). In addition, a low dose (625 cGy) of γ radiation at 5 Gy/min actually increased synaptic efficacy slightly, although statistically insignificantly. One might predict that at an even lower dose rate, such as the one used in the present study, reduction of synaptic efficacy would require even higher doses. Removal of the decrease in synaptic efficacy with lower dose rates may allow the expression of a distinct mechanism that increases synaptic efficacy.

An alternative explanation is that the increase and the decrease in synaptic efficacy are due to the same underlying mechanism that is biphasic in nature. We have hypothesized that the decrease is due to an oxidation of cellular proteins because oxidizing agents such as chloramine T and *n*-chlorosuccinimide can decrease synaptic efficacy in the same way as free radicals generated by peroxide and as exposure to γ irradiation (*20*). Oxidizing agents, radiation, and free radicals can impair calcium regulation by the mitochondrial, sarcoplasmic reticular, and/or plasma membranes, resulting in increased intracellular calcium concentration (*21–27*). Synaptic processes are markedly sensitive to calcium. A relatively small increase in presynaptic calcium levels can increase release of neurotransmitter and increase the synaptic potential. At higher levels of calcium, the divalent cation can block calcium influx and have other toxicological actions. Altered calcium regulation could explain both the increase in synaptic efficacy seen in the present study and the decrease seen with γ radiation at higher doses and dose rates.

There is precedent for biphasic changes in calcium-dependent processes. For example, in cardiac cells from the dog, the calcium-dependent spike is first prolonged and then blocked by exposure to free radicals generated either from peroxide or from dihydroxyfumarate (*28, 29*). At the synaptic contact between muscle and nerve (the endplate), exposure to mercury first increases and then decreases the synaptic potential (endplate potential) (*30*). The calcium ionophore X-537a, which allows influx of calcium into the presynaptic terminal, also has a biphasic effect on the endplate potential, first increasing and later decreasing the amplitude (*31*).

One might expect exposure to radiation *in vivo* to produce changes in neurons throughout the brain similar to those reported here for neurons in slices of hippocampus. Although it is difficult to predict from the present study the type of symptoms that might result from general nervous system dysfunction, the disorientation that results from radiation exposure would not be inconsistent. Since the hippocampus is thought to have a role in memory and learning, deficits in these functions may be prevalent if the damage is more specific to hippocampal neurons. *In vivo*, other factors also come into play. Humoral effects (e.g., increases in levels of prostaglandins or histamine) are likely to influence neuronal activity. In addition, reduced blood flow and alteration in the blood–brain barrier will affect brain function. Changes in glial cells can also alter the neuronal environment. The interactions of all of these factors must be considered before we can arrive at a full understanding of radiation damage to the nervous system.

The data presented in this report demonstrate that doses as low as 40 Gy X radiation can have direct effects on neuronal tissue *in vitro*. With the availability of an X-ray system that allows investigation into the radiation sensitivity of more complicated neuronal behavior than previously possible, future studies are likely to demonstrate neuronal damage at even lower doses.

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